2017

Assessing The Relationship Between Periodontal Disease And Subsequent Inflammatory Responses And Headache Disorders

Bryn E. Davis
University of South Carolina

ASSESSING THE RELATIONSHIP BETWEEN PERIODONTAL DISEASE AND SUBSEQUENT INFLAMMATORY RESPONSES AND HEADACHE DISORDERS

by

Bryn E. Davis

Bachelor of Science
Clemson University, 2015

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in Public Health in

Epidemiology

The Norman J. Arnold School of Public Health

University of South Carolina

2017

Accepted by:

Anwar Merchant, Director of Thesis

Alex McLain, Reader

Alan Decho, Reader

Cheryl L. Addy, Vice Provost and Dean of the Graduate School

ACKNOWLEDGEMENTS

This thesis uses data from a study supported by funding from National Institutes of Health contract N01WH32122, National Institutes of Health/National Institute of Dental and Craniofacial Research grants 1R01-DE13505 and DE04898, and United States Army Reserve Medical Corps grant DAMD 17-96-1-6319. I would like to thank Dr. Wacktawski-Wende for allowing us to use her data from the OsteoPerio WHI Ancillary study. I would also like to thank my committee, for their guidance and expertise during this process. Finally, I want to thank my cohort for their fellowship and assistance these last two years.

ABSTRACT

Headaches are the sixth leading cause of disability worldwide, and the third leading cause of disability amongst those aged 50 and older. Several headache disorders, including migraines, have been associated with nitric oxide production. It is widely accepted that the pain associated with headache disorders is due to the ability of nitric oxide to function as a vasodilator. While nitric oxide is a known vasodilator and has been linked to headache disorder, it is also produced in response to bacterial infections. Periodontal disease is the result of long term bacterial infections occurring in the gum line and nitric oxide is produced in response. Because periodontal disease incites the production of nitric oxide, it is biologically plausible that periodontal disease could be associated with headache disorders.

To investigate the relationship between periodontal disease and headache disorders, bivariate and ordinal logistic regressions were employed while controlling for age, education, income, physical activity level, smoking status, body mass index, and diet. The population consisted of 1206, postmenopausal women from the Women's Health Initiative.

While our study reports no significant association between periodontal disease and headache disorders, our study did provide evidence of a link between *Porphyromonas gingivalis*, an important microbe in periodontal disease

pathogenesis capable of inducing nitric oxide production, and migraines (Odds ratio = 2.252; 95% Confidence interval: 1.121 to 4.526).

P. gingivalis presence is positively associated with migraines among older white women after controlling for potential confounders. Further investigation into measures of oral health, oral microbes, and NO in relation to headache disorders is warranted.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND:

According to the Global Burden of Disease Survey released in 2010, (GBD2010), headaches are the 6th leading cause of disability worldwide, and the 3rd leading cause of disability amongst those aged 50 and older (Stephen S Lim‡, Theo Vos, Abraham D Flaxman, Goodarz Danaei et al., 2012). These findings illustrate the large impact headache disorders have on individuals and society, consequently, as a response, many organizations and alliances were established (Steiner, Stovner, & Birbeck, 2013). For example, the World Headache Alliance (WHA), a coalition of over 40 organizations formed with the aim "to reduce the burden of headache disorders throughout the world via sharing information among headache organizations and by increasing the awareness and understanding of headaches as a public health concern with profound social and economic impact" (WHA, 2017). Acknowledgement of the global burden experienced from headache disorders has called for more work in understanding all aspects of these disorders (Steiner et al., 2013), as a better understanding of factors that trigger headaches can help in prevention and reduce related disability.

One such study recently reported the possibility of a symbiotic relationship between oral commensal bacteria and humans through the salivary nitrate-nitrite-

nitric oxide pathway that could provoke migraines (Gonzalez et al., 2016). That study proposed that the symbiotic relationship between commensal, oral bacteria and salivary enzymes helps reduce nitrates ingested to nitric oxide (NO), which has been implicated in the pathophysiology of most headache disorders, including migraines, tension type headaches, and cluster headaches (D'andrea et al., 1994; Gruber et al., 2010; Olesen, 2008; Sarchielli et al., 1996; Shimomura, Murakami, Kotani, Ikawa, & Kono, 1999). Migraine pain felt in the temporomandibular, facial, and maxillary areas has been attributed to vascular dilatation (Tietien, 2009), and NO is an effective vasodilator. Because of NO's ability as a vasodilator, it is widely accepted that NO causes the pain suffered from migraines via the nociceptor activation resulting from the vascular dilatation of the dural and meningeal blood vessels (Schürk et. al., 2009). Consequently, nitrates, a precursor to NO, are commonly used as cardiac therapeutics (Thadani & Rodgers, 2006), with headaches being a common side effect of prescription nitrates (Bagdy, Riba, Kecskeméti, Chase, & Juhász, 2010). Furthermore, obstruction of nitric oxide synthases (NOS) has been shown to attenuate migraines without aura, tension-type headaches, and cluster headaches (Olesen, 2010).

The findings reported by Gonzalez and colleagues are the first contribution to headache etiology research suggesting oral microorganisms as the cause of headaches linked to NO (Gonzalez et al., 2016). The NIH funded OsteoPerio ancillary study of the Woman's Health Initiative (WHI) has collected data on oral microbial species identified in 1,256 postmenopausal women from

Buffalo, New York. Data from this study will allow us to further investigate the effect of oral microbes on headache disorders, however I propose a different causal pathway than Gonzalez and colleagues. I propose that bacterial infections associated with periodontal disease induce an inflammatory response, specifically the production of NO, that contributes to the vasodilation and subsequent pain suffered in headache disorders.

Periodontal diseases are chronic inflammatory infections associated with gram-negative bacteria (Slade, Offenbacher, Beck, Heiss, & Pankow, 2000). Severe periodontal disease slowly destroys the supporting tooth structures consisting of the gums and bone. Clinically, periodontal disease is classified based on the degree to which the supporting tooth structures are damaged. In addition to being a vasodilator, NO is also an oxidant and is produced by the immune system in response to bacterial infections (Mancinelli & McKay, 1983). The lipopolysaccharides (LPS) of several gram-negative bacteria (Eun Young Choi et al., 2011; Kim, Ha, Choi, Choi, & Choi, 2004; Pelt, Zimmermann, Ulbrich, & Bernimoulin, 2002; Skaleric, Gaspirc, McCartney-Francis, Masera, & Wahl, 2006; Hussain, Mckay, Gonzales-Marin, & Allaker, 2015; E. Y. Choi et al., 2007; Herath et al., 2016; Kato, Mikami, & Saito, 2001; Velsko et al., 2015; Chukkapalli et al., 2015) and the lipoteichoic acids of a gram-positive bacteria associated with periodontal disease have been shown, *in vivo*, to stimulate NO production by the immune system (English, Patrick, Orlicek, McCordic, & Shenep, 1996; Jian Jun Gao, Xue, Zuvanich, Haghi, & Morrison, 2001). Moreover, there is evidence of

increased NO production in inflamed, human gingival tissue (Kendall, Haase, Li, Xiao, & Bartold, 2000; Hirose et al. 2001).

While there is limited literature on the association between periodontal disease and headache disorders, there is plausible biological evidence supporting a causal link. The NO produced in response to periodontal disease could be triggering the pain suffered from headache disorders via vasodilation in the brain.

1.2 PURPOSE & SPECIFIC AIMS:

Aim 1: Assess the relationship between periodontal disease and headache disorders.

Hypothesis 1a**:** Severe or moderate periodontal disease, as defined by the Centers for Disease Control and Prevention (CDC) and American Academy of Periodontology (AAP), is positively associated with headache disorders compared with mild or no periodontal disease.

Hypothesis 1b: Increasing mean Clinical Attachment Level (CAL) is positively associated with headache disorders.

Aim 2: Assess the relationship between NO-production inducing bacteria and headache disorders.

Hypothesis 2a: Infection with bacteria in the dental plaque clinically shown to induce NO-production (model species: *Streptococcus sanguis*, *Prevotella intermedia*, *Campylobacter rectus, Porphyromonas gingivalis, Fusobacterium nucleatum*. and *Tannerella forsythensis*) is associated with headache disorders.

Hypothesis 2b: Infection with bacteria in the dental plague not clinically shown to induce NO-production (model species: *Capnocytophaga spp.*, and *Eubacterium saburreum*) is not associated with headaches.

CHAPTER 2

LITERATURE REVIEW

2.1 SEARCH METHODS:

A literature search was conducted via PubMed to locate studies evaluating the association between periodontal disease and headache disorders. Advanced search criteria limited the search results to studies published in the English language and performed on human subjects. No limits were set for publication date. I included the following search terms: (“headache” OR “migraine” OR “headache disorder” OR “migraine disorder” OR “cluster headache” OR “tension-type headache”) AND ("periodontal disease” OR "gingival disease” OR "gum disease” OR “periodontal disease" OR "gingivitis"). The PubMed search produced 28 articles and one additional article identified later, whose titles and abstracts were reviewed. Of those, six articles were read in their entirety to screen for inclusion. To be eligible for inclusion, the study had to assess the relationship between periodontal disease and any headache disorder. Because of the limited literature on this association, I also included pertinent case studies. Only one article assessed the relationship between periodontal disease and a headache disorder in an epidemiological study. Figure 2.1 is a flowchart of the literature review search.

2.2 FINDINGS:

Two studies (Table 2.1) were included in the literature review; one was a case-control study (Peskersoy, Peker, Kaya, Unalp, & Gokay, 2016) and the other a case report (Hoffman & McCulloch, 2013). Both studies had confirmed cases of oral health status via a dentist administered clinical assessment.

2.3 DISCUSSION:

The literature assessing the relationship between periodontal disease and headache disorders is extremely limited. Our literature search produced a single epidemiological study and one case report. Peşkersoy and colleagues recently published the results of their multi-center, parallel, case-controlled clinical study assessing the relationship between migraine disorders and oral comorbidities. They found that tooth wear and abrasion were more frequent in patients with migraines (76%, $P < 0.05$). Furthermore, the mean number of decayed, missing or filled teeth (DMFT) (mean 3.79 vs. 2.71, $P < 0.05$) and gingival plaque index scores (mean 1.64 vs. 1.23, $P < 0.05$) showed significant differences between those with and without migraines (Peskersoy et al., 2016). However, they proposed that the oral comorbidities were the result of their migraine disorder and not vice versa, yet this case-controlled study did not establish temporality thus limiting the ability to determine causality. The study does, however, provide evidence of an association between oral health status and headache disorders that warrants further examination.

Furthermore, the case report found in our literature review was on a 60-year-old man with a 7-year history of cluster headaches. The patient sought

treatment from an oral surgeon for pain in the left upper second molar ipsilateral upon which the doctor discovered severe infection, decay, and inflammation while extracting the tooth. Removal of the infected tooth resulted in the attenuation of his cluster headaches. However, the authors propose that nerve involvement was the cause of the cluster headaches (Hoffman & McCulloch, 2013). No measures or rankings of periodontal disease were reported for this case report.

2.4 FUTURE DIRECTIONS:

Future studies investigating the etiology of headache disorders should focus on oral comorbidities. While the literature investigating the relationship between periodontal disease and headache disorders is limited, the clinical evidence supports the biological plausibility outlined in the background. Therefore, my investigation into the relationship between periodontal disease and headache disorders would contribute to the limited existing literature.

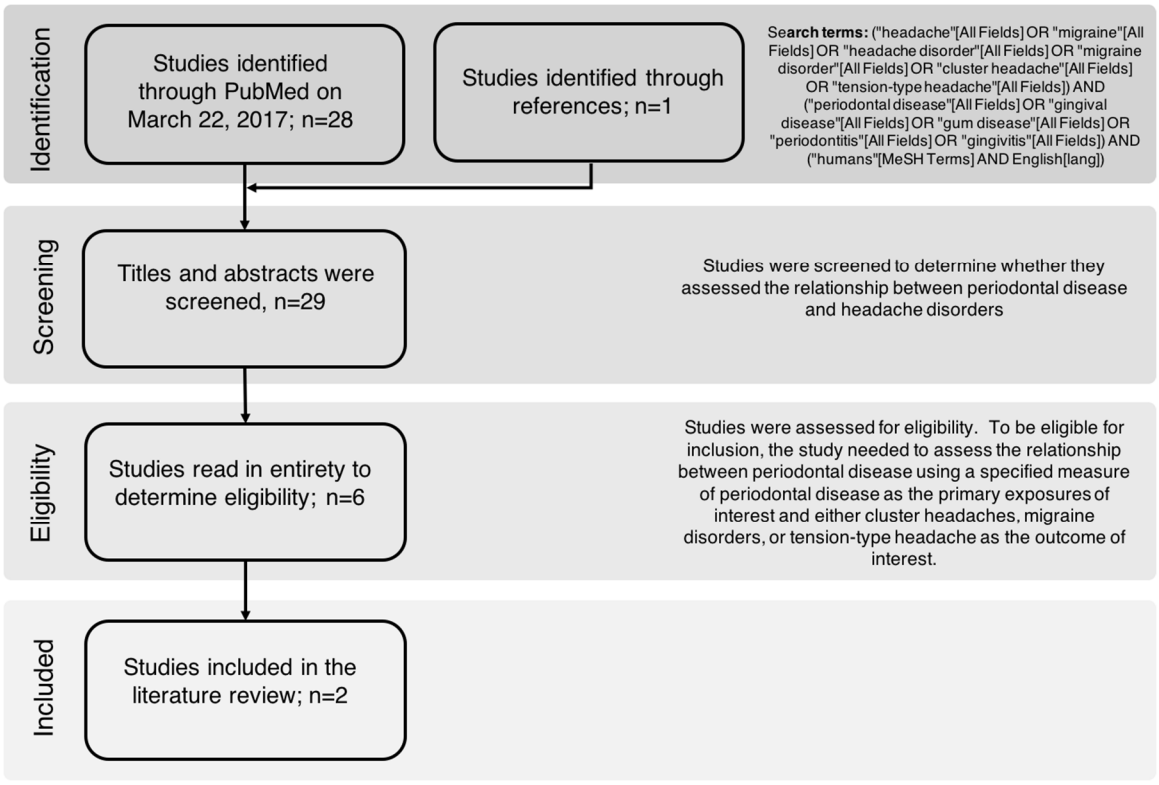

Figure 2.1: Flowchart of literature review

Table 2.1: Epidemiological and clinical studies investigating the association between periodontal disease and headache disorders

First author, year(s), place	Study design and sample size	Measure of periodontal disease	Outcome variable(s)	Control variables	Main findings
Peskersoy et al. 2016, Turkey	Case-control, n=2001	DMFT and gingival plaque index	Migraine	Adjustment was made for individual's sex, age, systemic conditions, existence of comorbidities, and overall dental health and care status.	Tooth wear and abrasion was more frequent in patients with migraine (76%, $P < 0.05$). Furthermore, the DMFT scores (mean 3.79 vs. 2.71, $P < 0.05$) and gingival plaque index scores (mean 1.64 vs. 1.23, $P < 0.05$) showed significant differences between the groups.
Hoffman & McCullough 2013, USA	Case report	Physician inspection revealed signs of decay and periodontitis. No official diagnosis for periodontal disease was stated. No measures of periodontal disease were reported.	Cluster headache	N/A	"A 60-year-old man with a 7-year history of cluster headaches was seen by an oral surgeon for evaluation of pain in the left upper second molar ipsilateral to the side affected by the headaches. During extraction of the tooth, infection, decay, and inflammation were discovered. Since the extraction in November 2008, the patient has experienced one episode of cluster headaches as of April 2013."

CHAPTER 3

METHODS

3.1 STUDY POPULATION:

Participants were recruited from the WHI OS, during their year 3 visit, to participate in the Buffalo Osteoporosis and Periodontal Disease Study (OsteoPerio Study), an ancillary study of the Women's Health Initiative (WHI) Observational Study (OS) seeking to "examine the association between osteoporosis, oral bone loss, and periodontal disease in a well-characterized cohort of postmenopausal women" (Sahli et al., 2015). The OsteoPerio study has previously been reviewed and approved by the Health Sciences Institutional Review Board at the University at Buffalo, and all participants provided signed informed consent. Participants were offered an incentive of a free bone density scan and an oral examination (Brennan et al., 2007). Procedures for study recruitment, retention, and data collection have been previously described (Wactawski-Wende et al., 2005; Brennan et al., 2007; Sahli et al., 2015). My study population is comprised of 1,206 female participants; the population was reduced from 1,256, as participants with missing values for all exposures and outcomes of interest were excluded.

3.2 EXPOSURE OF INTEREST FOR AIM I:

Participants were offered free oral exams as incentive for participation in the OsteoPerio ancillary study. This occurred during their year three clinical

assessment. This included assessing the presence of decaying or filled teeth and accounting for each missing tooth via self-report and several measures of periodontal disease were taken. Moreover, samples of subgingival plaque were taken from each participant (Brennan et al., 2007). Pocket depth (PD) and mean Clinical Attachment Level (CAL) were two of the measures used to classify participants' severity of periodontal disease. PD is a measure of the distance from the gingival margin to the bottom of the pocket and is a useful measure of periodontal disease. Similarly, CAL is a measure of the distance from the cement-enamel junction to the bottom of the pocket (American Academy of Periodontology, 2015).

To address the first hypothesis of Aim 1, participants were classified as having no or mild, moderate, or severe periodontal disease based on the CDC and AAP rankings (Page & Eke, 2007), and recorded as 0, 1, or 2, respectively. Mild periodontal disease was defined as have two or more interdental sites with pocket depth (PD) $\geq$ 4 mm and two or more interdental sites with CAL $\geq$ 3 mm or having PD $\geq$ 5 mm on any tooth. Moderate periodontal disease was defined as having two or more interdental sites on different teeth with CAL $\geq$ 4 mm or having PD $\geq$ 5 mm on more than one interdental site on different teeth. Severe periodontal disease was classified as having one or more interdental sites with PD $\geq$ 5 mm and two or more interdental sites with CAL $\geq$ 6 mm (Page & Eke, 2007).

Additionally, information gathered from the oral examination was used calculate a mean CAL for each participant. To make comparisons between

various levels of mean CAL, participants were divided into four groups, the quartiles of mean CAL. Quartile 1 (Q1) is comprised of participants with a mean CAL less than or equal to 1.95. Quartile 2 (Q2) is comprised of participants with a mean CAL greater than 1.95 and less than or equal to 2.27. Quartile 3 (Q3) is comprised of participants with a mean CAL greater than 2.27 and less than or equal to 2.66. Lastly, Quartile 4 (Q4) is comprised of participants with a mean CAL greater than 2.66. Additionally, mean CAL was divided into quartiles within all logistic regression models.

3.3 EXPOSURE OF INTEREST FOR AIM II:

As part of the oral examination received by participants in OsteoPerio ancillary study, subgingival plaque samples were taken via the paper point technique (Brennan et al., 2007). Samples were taken from up to 12 teeth previously dried with cotton to reduce contamination. The following species were selected for this study and detected via immunofluorescence: *Streptococcus sanguis*, *Prevotella intermedia*, *Campylobacter rectus, Porphyromonas gingivalis, Fusobacterium nucleatum*. *Tannerella forsythensis*, *Capnocytophaga spp.*, and *Eubacterium saburreum.* Methods have been previously described in detail (Brennan et al., 2007).

For this study, the bacterial species were divided into two groups. A literature search was conducted for clinical evidence of inducing NO production for each of the targeted bacterial species. Results from the search are recorded in Table 3.1. Group 1 consists of bacterial species that may induce NO production, as supported by literature, and Group 2 consists of the remaining

bacterial species not known to induce NO production. Group 1 includes *S. sanguis*, *P. intermedia*, *C. rectus, P. gingivalis, F. nucleatum*, and *T. forsythensis*. Group 2 includes the remaining species as there is no literature reporting the ability of *Capnocytophaga* spp. or *E. saburreum* to induce NO production. All species were recoded as either present (1) or absent (0); therefore, an additional variable “Infection” was created to illustrate the number of Group 1 bacteria identified in each participant. All participants who were infected with two or less Group 1 bacteria were considered to have no or mild infection. Participants who were infected with three or four Group 1 bacteria were considered to have moderate infection; and participants who were infected with five or six Group 1 bacteria were considered to have severe infection.

3.4 OUTCOMES OF INTEREST:

3.4.1 Migraines

During their baseline visit for the WHI OS, participants were asked “Has a doctor told you that you have any of the following conditions or have you had any of the following procedures?” and were provided a list that included migraine headaches. Participants responses were coded as absent (0) or present (1). Because the presence of migraines was recorded as absent or present, with no information about type of migraine, bivariate logistic regression was used for all models assessing the relationship between migraines and our exposures of interest.

3.4.2 Headaches

During their third-year visit, participants were given a list of symptoms, one of which being headaches or migraines, and asked “for each item, mark the one… [response] that best describes how bothersome the symptom was during the past 4 weeks for you”. The options were “Symptom did not occur”, “Symptom was mild” defined as symptom did not interfere with usual activities, “Symptom was moderate” defined as symptom interfered somewhat with usual activities, or “Symptom was severe” defined as symptom was so bothersome that usual activities could not be performed. The response to this question was used as an indicator of the presence of any headache disorder, and because the response was recorded ordinally, ordinal or cumulative logistic regression was implemented in all models examining the relationship between our exposures of interest and headache disorders.

3.5 POTENTIAL CONFOUNDERS:

The following variables were identified as potential confounders via a literature search: age, education, income, race, body mass index (BMI), anxiety, depression, smoking, physical activity, cholesterol, medication use, blood pressure, and eating behaviors (Aamodt, Stovner, Midthjell, Hagen, & Zwart, 2007; Bond, Roth, Nash, & Wing, 2011; Buse, Silberstein, Manack, Papapetropoulos, & Lipton, 2013; Cappy, Lucas, Catteau-Jonard, & Robin, 2015; Fagernæs et al., 2015; Heckman & Britton, 2015; Martin & Vij, 2016; Stovner et al., 2007; Thadani & Rodgers, 2006; Varkey, Hagen, Zwart, & Linde, 2008; Younger, 2016). However, data collected on cholesterol were too incomplete to

include in the analysis and the population was mostly white (97.4%) thus race was also excluded from the analysis. The remaining potential confounders were controlled for in each model to create the most theoretically based model. Refer to Figure 3.1, a Directed Acyclic Graph (DAG) illustrating the association between periodontal disease and headache disorders and the previously mentioned potential confounders. Additionally, Table 3.2 lists each variable treated as a potential confounder when building the model, literature justifying why the variable was considered a confounder, and how the variable was recorded.

3.6 STATISTICAL ANALYSIS:

3.6.1 Demographic differences

Participants were ranked by periodontal disease status as defined by the CDC and AAP. Comparisons were made between those with no or mild periodontal disease versus those with moderate or severe periodontal disease. Differences in continuous variables are recorded as the mean and standard deviation (SD) and assessed via the Student *t* test. Categorical variables were assessed via the Pearson Chi-square test or the Fischer's Exact test when appropriate.

Similarly, differences between participants at the four quartiles of mean CAL are recorded in Table 5. Comparisons were made between Q1, Q2, Q3, and Q4 of mean CAL. Differences in continuous variables are recorded as the mean and standard deviation (SD) and assessed via the one-way ANOVA test.

Categorical variables were assessed via the Pearson Chi-square test or the Fischer's Exact test when appropriate.

Furthermore, comparisons were made between those with no or mild, moderate, or severe Group 1 bacterial infections. Differences in continuous variables are recorded as the mean and standard deviation (SD) and assessed via one-way ANOVA test. Categorical variables were assessed via the Pearson Chi-square test or the Fischer's Exact test when appropriate.

Lastly, comparisons were made between the participants with no, one, or two Group 2 bacteria present. Differences in continuous variables are recorded as the mean and standard deviation (SD) and assessed via the one-way ANOVA test. Categorical variables were assessed via the Pearson Chi-square test or the Fischer's Exact test when appropriate.

3.6.2 Models

3.6.2.1 Migraines

To address the hypotheses stated in Aim 1, periodontal disease status was defined two ways, by CDC/AAP rankings and by mean CAL quartiles. To address Hypothesis 1a, bivariate logistic regression was implemented to determine the odds of having suffered migraines for those with moderate or severe periodontal disease compared to those with no or mild periodontal disease while controlling for age at enrollment, history of using birth control, cardiac therapeutic usage, caffeine intake in mg, and alcohol servings per week. Furthermore, an additional bivariate logistic regression was used to determine the odds of having suffered migraines for those with mean CAL in quartile 2, 3,

and 4 compared to quartile 1 to address Hypothesis 1b. Similarly, this model also controlled for age at enrollment, history of using birth control, cardiac therapeutic usage, caffeine intake in mg, and alcohol servings per week.

To address both hypotheses stated in Aim 2, a final bivariate logistic regression model was employed to determine the odds of having suffered a migraine for those with moderate or severe infection of Group 1 bacteria compared to those with no or mild infection of Group 1 bacteria while controlling for the presence or absence of Group 2 bacteria, age at enrollment, history of using birth control, cardiac therapeutic usage, caffeine intake in mg, and alcohol servings per week. It is necessary to control for the presence of Group 2 bacteria within the model assessing the relationship between Group 1 bacteria and migraines; therefore, we only need the single model to address both hypotheses from Aim 2. Additional bivariate logistic regression models were used to assess the relationship between each Group 1 bacterial species and migraines. All six models controlled for age at enrollment, history of using birth control, cardiac therapeutic usage, caffeine intake in mg, and alcohol servings per week.

3.6.2.2 Headache disorders

Methods for assessing the odds of having suffered a headache disorder for each hypothesis are like the previously mentioned methods for assessing the odds of having suffered a migraine, with a few exceptions. Because participants were asked if they had suffered no or mild, moderate, or severe pain, ordinal regression was employed to determine the odds of having suffered a headache disorder for all exposures. All models controlled for age at enrollment, history of

using birth control, cardiac therapeutic usage, blood pressure, and alcohol servings per week.

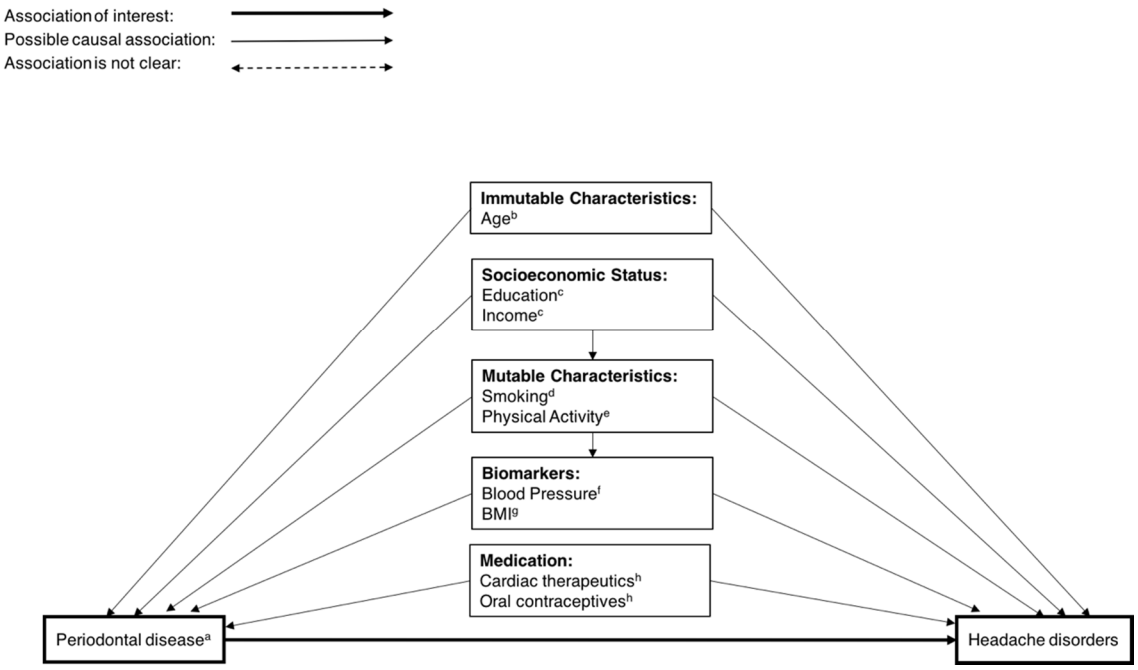

Figure 3.1: Directed Acyclic Graph (DAG) of the association between periodontal disease and headache disorders

a. **Sources:** Katz et al., 2002
b. **Sources:** Mirowsky & Ross, 1992; Jorm, 2000; Twenge, 2000; Stevens et al., 1998; Franklin et al., 1997; Schaefer et al., 1994
c. **Sources:** Leite et al. ,2017; Bodnar et al., 2017; Green & Benzeval,d 2013; Wardle & Steptoe, 2003; Hagen et al., 2002; Martikainen & Marmot 2017; Colhoun, Hemingway, & Poulter 1998; Winkleby, et al. 1998
d. **Sources:** Aamodt, Stovner, Midthjell, Hagen, & Zwart, 2007; Primatesta et al., 2001; Gepner et al., 2011; Tomar & Asma, 2000
e. **Sources:** Varkey, Hagen, Zwart, & Linde, 2008; Stefanick et al., 1998; Cornelissen & Smart, 2013; WHO 2013; Bawadi et al., 2011
f. **Sources:** Fagernæs et al., 2015; Prospective Studies Collaboration, 2007
g. **Sources:** Bond, Roth, Nash, & Wing, 2011; Brown et al., 2000; Faheem et al., 2010
h. **Sources:** Thadani & Rodgers, 2006; Cappy, Lucas, Catteau-Jonard, & Robin, 2015

T**able 3.1** Group 1 and Group 2 bacterial species	
Bacteria spp. and characteristics	**Support**
Group 1: NO production inducting bacteria	
Streptococcus sanguis	While periodontal disease is typically associated with gram-negative bacteria, the lipoteichoic acids from *S. sanguis* has been shown to stimulate NO production from murine and C3H/HeJ macrophages (English, Patrick, Orlicek, McCordic, & Shenep, 1996; Jian Jun Gao, Xue, Zuvanich, Haghi, & Morrison, 2001).
Prevotella intermedia	*P. intermedia* lipopolysaccharide (LPS) has been shown to stimulate the release of NO (Eun Young Choi et al., 2011; Kim, Ha, Choi, Choi, & Choi, 2004; Pelt, Zimmermann, Ulbrich, & Bernimoulin, 2002; Skaleric, Gaspirc, McCartney-Francis, Masera, & Wahl, 2006)
Campylobacter rectus	*C. rectus* LPS has been shown to induce NO production in oral keratinocytes (Hussain, Mckay, Gonzales-Marin, & Allaker, 2015)
Porphyromonas gingivalis	*P. gingivalis* lipid A-associated proteins and LPS can induce nitric oxide production (E. Y. Choi et al., 2007; Herath et al., 2016).
Fusobacterium nucleatum	Live *F. nucleatum* can induce NO production in murine macrophages (Kato, Mikami, & Saito, 2001).
Tannerella forsythensis **(formally known as *T. forsythia*)**	There is evidence that a polymicrobial consortium including *Tannerella forsythia* was shown to increase NO production (Velsko et al., 2015). **However, there are conflicting reports on whether *T. forsthensis* induces NO production** (Chukkapalli et al., 2015)**.**
Group 2: Control bacteria not considered to induce NO production	
Capnocytophaga spp.	No evidence of inducing NO production
Eubacterium saburreum	No evidence of inducing NO production

Table 3.2: Potential confounders for the association between periodontal disease and headache disorders

Variable	Justification
Age at baseline	Will include to control for age as a potential confounder and to prevent any age affects.
Education	When defining SES by educational level or type of occupation, low status was associated with increased risk of frequent and chronic headache at follow-up. (Hagen et al., 2002)
Income	Previous studies found that risk of headache decreased with increasing income, but only among men (Hagen et al., 2002). It's still important to see how income affects headaches in this exclusively female population.
BMI	Evidence indicates a possible link between migraine and obesity, with the latter either exacerbating headache activity in people currently suffering from migraine or possibly increasing the risk for having migraine. (Bond et al., 2011)
Depression variable	Migraine is comorbid with several psychiatric disorders, including depression, anxiety disorders, and PTSD. (Buse et al., 2013)
Smoking	Passive smoking was associated with higher headache prevalence. (Aamodt et al., 2007)
Physical Activity	A study reported that individuals reporting headaches were typically less active (Varkey et al., 2008).
Medication use: Cardiac therapeutics	Nitrates are effective cardiac agents; however headaches are the most common consequence (Thadani & Rodgers, 2006).
Medication use: Oral contraceptive	Oral contraceptives have been associated with migraine disorders (Cappy et al., 2015).
Blood pressure	Two large-scale population-based cohort studies, HUNT2 and HUNT3 reported an inverse association between blood pressure and headaches (Fagernæs et al., 2015).
Dietary intake: Caffeine	Caffeine withdrawal has been reported to trigger headaches (Martin & Vij, 2016). Headaches are associated with alcohol overuse (Aamodt et al., 2007) (Martin & Vij, 2016). Caffeine withdrawal have the strongest evidence for triggering attacks of headache (Martin & Vij, 2016)

CHAPTER 4

RESULTS

4.1 POPULATION DEMOGRAPHICS:

Population characteristics are shown in Tables 4.1-10. Table 4.1 displays our population characteristics by each exposure level that addresses our hypotheses. The majority of the study population suffers from periodontal disease with 16.25% suffering from severe periodontal disease and 57.05% suffering from moderate periodontal disease. Most participants (51.49%), were infected with one or two Group 1 bacteria, and no Group 2 bacteria (50.58%). Results are shown in Table 4.

As shown in Tables 4.2 and 4.3, differences between participants with moderate or severe periodontal disease were older, more likely to be former or current smokers, were less physically active, and were more likely to be infected with *P. intermedia, T. forsythensis, E. saburreum, C. rectus,* and *P. gingivalis* than participants with no or mild periodontal disease. No statistically significant differences were observed between participants with moderate or severe periodontal disease versus participants with no or mild periodontal disease with regards to BMI, history of birth control usage, cardiac therapeutic usage, diastolic blood pressure, caffeine intake, alcohol servings per week, and the presence of *S. sanguis*, *Capnocytophaga* spp. or *F. nucleatum*.

Differences between the quartiles of mean CAL are also found in Tables 4.4-4.6. Participants in the fourth quartile are older, more likely to be current smokers, less physically active, and are more likely to be infected with *P. intermedia, T. forsythensis*, *E. saburreum*, *C. rectus,* and *P. gingivalis.* Additionally, participants in the third and fourth quartiles drank more caffeine than participants in the first and second quartiles. No significant differences were seen between the four quartiles in regard to total family income, education, BMI, history of birth control usage, cardia therapeutic usage, diastolic blood pressure, alcohol servings per week, or infection with *S. sanguis*, *Capnocytophaga sp.*, and *F. nucleatum.*

As see in Tables 4.7 and 4.8, participants with severe infections of Group 1 bacteria were more likely to be overweight, less likely to be current smokers, drank more caffeine, and were more likely to be about of the fourth quartile of Mean CAL. Similarly, participants with more severe Group 2 infections were also more likely to be overweight (Tables 4.9-4.10). Differences between the varying degrees of infections for Group 1 and Group 2 bacteria are recorded in Table 6.

4.2 MODEL RESULTS:

4.2.1 Migraines

Results from crude and adjusted models assessing the specific aims and all hypotheses for migraines is displayed in Table 4.11. To address Aim 1, bivariate logistic regression models were implemented to assess the relationship between periodontal disease and migraines; periodontal disease was defined two ways, by CDC/AAP rankings and by Mean CAL. Severe or moderate periodontal

disease defined by CDC/AAP rankings was not associated with migraines in both unadjusted (OR = 0.812, 95% CI: 0.544, 1.213) and adjusted (OR = 0.811, 95% CI: 0.530, 1.241) models. Similarly, periodontal disease classified by quartiles of Mean CAL was not associated with migraines in both unadjusted and adjusted models when comparing the second and third quartiles to the first quartile. The unadjusted and adjusted OR for participants in the second quartile of Mean CAL was 1.109, 95% CI: 0.673, 1.827 and 1.071, 95% CI: 0.628, 1.828, respectively. When comparing participants in the third quartile of Mean CAL to participants in the first quartile of Mean CAL, the odds of suffering from a migraine were 1.746, 95% CI: 1.000, 3.048 in the crude model and 1.638, 95% CI: 0.904, 2.966, in the adjusted model. However, for participants in the fourth quartile, the unadjusted association between Mean CAL was not associated with migraines in the crude (unadjusted OR = 1.065, 95% CI 0.648, 1.749) or adjusted models (adjusted OR = 0.930, 95% CI: 0.546, 1.584). Both adjusted models controlled for age, education, income, BMI, smoking status, physical activity, history of birth control, cardiac therapeutic usage, diastolic blood pressure, alcohol consumption, and caffeine consumption.

To address hypotheses from Aim 2, a bivariate logistic regression model was implemented to assess the relationship between Group 1 and 2 bacteria and migraines. The adjusted model controlled for all the above-mentioned covariates. The relationship between Group 1 bacterial species and migraines was assessed two ways, via an infection ranking and by assessing the relationship between the presence of each individual bacterial species. More information on the bacterial

species of interest and the infection ranking is in section 3.3. Moderate and severe infection with Group 1 bacteria approached positive associations with migraines in unadjusted and adjusted models, but did not reach statistical significance. Participants with moderate infection compared to those with no or mild infection had an unadjusted OR of 1.622, 95% CI: 0.992, 2.652 and adjusted OR 1.511, 95% CI: 0.879, 2.599. Also, participants with severe infection compared to those with no or mild infection have unadjusted OR 1.589, 95% CI: 0.673, 3.754 and adjusted OR 1.293, 95% CI: 0.508, 3.288. The presence of *P. gingivalis* was positively associated with migraines in unadjusted (OR = 2.109, 95% CI: 1.082, 4.110), and adjusted (OR = 2.252, 95% CI: 1.121, 4.526) models. Other individual Group 1 bacterial species were not associated with migraines.

Infection with one or two Group 2 bacteria, the control bacteria, were not associated with migraines. The odds of suffering from migraines for those with a single infection of Group 2 bacteria was 0.821, 95% CI: 0.545, 1.236 in the unadjusted model and 0.776, 95% CI: 0.498, 1.207 in the adjusted model. Infection with two Group 2 bacteria resulted in an unadjusted OR of 1.455, 95% CI: 0.852, 2.484 and adjusted OR of 1.363, 95% CI: 0.756, 2.458. Both Group 2 bacterial species, *Capnocytophaga sp.* and *E. saburreum*, were positively, yet insignificantly associated with migraines in crude and adjusted analyses.

4.2.2 Headaches

Similar analyses were performed to also assess the relationship between our exposures and headache disorders; however, the variable for headache disorders was recorded as an ordinal variable with four options. As such, ordinal

logistic regression was implemented and results for all crude and adjusted models related to headache disorders were recorded in Tables 4.12-4.14. The odds of suffering from mild, moderate, and severe headache symptoms are recorded in Tables 4.12, 4.13, and 4.14, respectively. From these data, we must conclude that periodontal disease was not associated with headaches. Severe or moderate periodontal disease as defined by the CDC/AAP rankings was not significantly associated with any level of headache pain. For participants suffering from moderate or severe periodontal disease, the odds of suffering mild headache symptoms were 0.865, 95% CI: 0.699, 1.119 in the crude model and 0.758, 95% CI: 0.336,1.667 in the adjusted model. For participants suffering from moderate or severe periodontal disease the odds of suffering from moderate headache symptoms was 0.737, 95% CI: 0.497,1.091 in the crude model and 0.728, 95% CI: 0.483,1.098 in the adjusted model. For participants suffering from moderate or severe periodontal disease the odds of suffering from severe headache symptoms was 0.768, 95% CI: 0.358,1.650 in the unadjusted model and 0.758, 95% CI: 0.336, 1.710 in the adjusted model. The adjusted model controlled for age, education, income, BMI, smoking status, physical activity, history of birth control, cardiac therapeutic usage, diastolic blood pressure, alcohol consumption, and caffeine consumption.

To address Hypothesis 2 from Aim 1, ordinal logistic regression was implemented to assess the relationship between mean CAL and suffering various levels of headache pain. Again, our data suggests no relationship between periodontal disease and headaches. For participants in the second quartile of

Mean CAL, the odds of suffering from mild headache symptoms was 1.015, 95% CI: 0.735, 1.402 in the unadjusted model and 0.986, 95% CI: 0.701,1.387 in the adjusted model. For participants in the third quartile of Mean CAL, the odds of suffering from moderate headache symptoms was 0.699, 95% CI: 0.402,1.112 in the unadjusted model and 0.614, 95% CI: 0.356,1.059 in the adjusted model. Lastly, for participants in the fourth quartile of Mean CAL, the odds of suffering from severe headache symptoms was 0.518, 95% CI: 0.189,1.419 in the unadjusted model and 0.510, 95% CI: 0.168,1.544 in the adjusted model. The adjusted model controlled for age, education, income, BMI, smoking status, physical activity, history of birth control, cardiac therapeutic usage, diastolic blood pressure, alcohol consumption, and caffeine consumption.

To address both hypotheses from Aim 2, ordinal logistic regression was used to assess the relationship between Group 1 and 2 bacteria and headache disorders. All adjusted models addressing Aim 2 controlled for age, education, income, BMI, smoking status, physical activity, history of birth control, cardiac therapeutic usage, diastolic blood pressure, alcohol consumption, and caffeine consumption. To address first hypothesis in Aim 2, ordinal logistic regression was implemented to assess the relationship between Group 1 bacteria and headache disorders. Moderate infection with Group 1 bacteria was not significantly associated with any level of headache pain. For participants with moderate infection of Group 1 bacteria, the odds of suffering from mild headache symptoms were 0.855, 95% CI: 0.649,1.125 in the unadjusted model and 0.844, 95% CI: 0.623,1.144 in the adjusted model. For participants with moderate

infection of Group 1 bacteria, the odds of suffering from moderate headache symptoms was 0.875, 95% CI: 0.561,1.366 in the unadjusted model and 0.819, 95% CI: 0.507,1.322 in the adjusted model. Lastly, for participants with moderate infection of Group 1 bacteria, the odds of suffering from severe headachy symptoms was 0.819, 95% CI: 0.507,1.322 in the unadjusted model and 0.726, 95% CI: 0.269,1.956 in the adjusted model. Similarly, severe infection with Group 1 bacteria was insignificantly associated with all levels of headache pain. For participants with severe infection of Group 1 bacteria, the odds of suffering from mild headache symptoms was 0.801, 95% CI: 0.499,1.287 in the unadjusted model and 0.855, 95% CI: 0.527,1.488 in the adjusted model. For participants with severe infection of Group 1 bacteria, the odds of suffering from moderate headache symptoms was 0.422, 95% CI: 0.151,1.181 in the unadjusted model and 0.458, 95% CI: 0.161,1.306 in the adjusted model. For participants with severe infection of Group 1 bacteria, the odds of suffering from severe headache symptoms was 0.431, 95% CI: 0.058,3.229 in the unadjusted model and 0.530, 95% CI: 0.070,4.024 in the adjusted model. None of the Group 1 species were significantly associated with any symptoms suffered from headaches.

Infection with one Group 2 bacterium was insignificantly associated with suffering from mild headache symptoms in the unadjusted (unadjusted OR = 0.986, 95% CI: 0.755,1.287) and adjusted models (adjusted OR = 1.016, 95% CI: 0.764,1.352). Conversely, infection with one Group 2 bacterium was significantly associated with mild headache symptoms in both the unadjusted and adjusted

models with OR of 1.672, 95% CI: 1.111,2.516 and 1.753, 95% CI: 1.139,2.697, respectively. Likewise, infection with one Group 2 bacterium was significantly associated with severe headache symptoms in both unadjusted and adjusted models with OR of 2.856, 95% CI: 1.281,6.368 and 3.169, 95% CI: 1.310,7.663, respectively. Infection with two Group 2 bacteria was not significantly associated with suffering from mild headache symptoms in the unadjusted and adjusted models with ORs of 0.902, 95% CI: 0.670,1.215 and 0.973, 95% CI: 0.695,1.363, respectively. However, infection with two Group 2 bacteria was not significantly associated with suffering from moderate and severe headache symptoms in crude and adjusted models. Infection with two Group 2 bacteria resulted in OR of 1.051, 95% CI: 0.635,1.740 and 1.168, 95% CI: 0.683,1.998 in the crude and adjusted models respectively for suffering from moderate headache symptoms. Infection with two Group 2 bacteria resulted in OR of 1.202, 95% CI: 0.407,3.555 and 1.578, 95% CI: 0.507,4.910 in the crude and adjusted models respectively for those suffering from severe headache symptoms. No individual species of Group 2 bacteria was significantly associated with any level of headache symptoms.

Table 4.1 Population demographics for WHI OsteoPerio ancillary study participants for each exposure of interest

Variable	Definition	n (%)
CDC/AAP Rankings of Periodontal Disease		
No or mild disease	Two or more interdental sites with pocket depth (PD)4 mm and two or more interdental sites with CAL 3 mm or having PD5 mm on any tooth.	322 (26.70)
Moderate disease	Two or more interdental sites on different teeth with CAL4 mm or having PD 5 mm on more than one interdental site on different teeth.	688 (57.05)
Severe disease	One or more interdental sites with PD5 mm and two or more interdental sites with CAL6 mm.	196 (16.25)
Mean CAL Quartiles		
Q1	Mean CAL less than or equal to 1.95	296 (24.54)
Q2	Mean CAL greater than 1.95 and less than or equal to 2.27	304 (25.21)
Q3	Mean CAL greater than 2.27 and less than or equal to 2.66	302 (25.04)
Q4	Mean CAL greater than 2.66	304 (25.21)
Group 1 Bacteria		
No disease	No Group 1 bacteria	229 (18.99)
Mild disease	1 or 2 Group 1 bacteria	621 (51.49)
Moderate Disease	3 or 4 Group 1 bacteria	278 (23.05)
Severe Disease	5 or 6 Group 1 bacteria	78 (6.47)
Group 2 Bacteria		
No Group 1 bacteria present	No Group 2 bacteria	610 (50.58)
1 Group 1 bacterium present	1 Group 2 bacteria	346 (28.69)
2 Group 1 bacteria present	2 Group 2 bacteria	250 (20.73)

Table 4.2. Population categorical characteristics for WHI OsteoPerio ancillary study participants by CDC/AAP rankings

	Total (n = 1206)	CDC/AAP Periodontal Disease Definition		
		No/Mild (n=322)	Moderate/ Severe (n=884)	*P* value
Age at enrollment				
<70 years	802 (66.50)	234 (72.67)	568 (64.25)	0.0061
≥70 years	402 (33.50)	88 (27.33)	316 (35.75)	
Total family income				
<$50,000	756 (62.69)	186 (57.76)	570 (64.48)	0.0329
≥$50,000	450 (37.31)	136 (42.24)	314 (35.52)	
Education				
High School	249 (21.01)	57 (17.92)	192 (22.15)	0.2225
College	530 (44.73)	143 (44.97)	387 (44.64)	
Post-College	406 (34.26)	118 (37.11)	288 (33.22)	
BMI				
Underweight/ Normal (< 24.9 kg/m^2)	541 (44.86)	137 (42.55)	404 (45.70)	0.6041
Overweight (25.0- 29.9 kg/m^2)	411 (34.08)	113 (35.09)	298 (33.71)	
Obese (≥30.0 kg/m^2)	254 (21.06)	72 (22.36)	182 (20.59)	
Cigarette smoking				
Never	635 (52.65)	190 (59.01)	445 (50.34)	0.0202
Former	531 (44.03)	125 (38.82)	406 (45.93)	
Current	40 (3.32)	7 (2.17)	33 (3.73)	
Birth control usage	456 (37.81)	126 (39.13)	330 (37.33)	0.5685
Cardiac therapeutic usage	29 (2.41)	7 (2.17)	22 (2.49)	0.4564
Bacterial sp., No. (%)				
S. sanguis	713 (59.12)	187 (58.07)	526 (59.50)	0.6555
P. intermedia	515 (42.70)	119 (36.96)	396 (44.80)	0.0149
T. forsythensis	453 (37.65)	74 (22.98)	379 (42.87)	<.0001
Capnocytophaga sp.	450 (37.31)	112 (34.78)	338 (38.24)	<.0001
E. saburreum	168 (13.93)	76 (23.60)	320 (36.20)	<.0001
C. rectus	202 (16.75)	43 (13.35)	159 (17.99)	0.0567
P. gingivalis	175 (14.51)	25 (7.76)	150 (16.97)	<.0001
F. nucleatum	168 (13.93)	38 (11.80)	130 (14.71)	0.1975

Table 4.3. Population continuous characteristics for WHI OsteoPerio ancillary study participants by CDC/AAP rankings

		CDC/AAP Periodontal Disease Definition		
	Total (n = 1206)	**No/Mild (n=322)**	**Moderate/ Severe (n=884)**	***P* value**
Age at enrollment, mean±SD, y	66.47±6.96	65.32±6.87	66.90±6.95	0.5743
Physical activity, mean±SD, MET hours per week	14.44±14.31	15.97±16.30	13.89±13.48	0.0270
Diastolic BP, mean±SD, mmHg	70.78±8.96	71.03±8.95	70.70±8.96	0.5743
Caffeine intake, mean±SD, dietary caffeine mg	167.86±127.43	156.4±116.5	172.0±131.0	0.0662
Alcohol intake, mean±SD, servings per week	2.69±1.75	2.48±4.20	2.76±4.93	0.3746

Table 4.4. Population categorical characteristics for WHI OsteoPerio ancillary study participants by Mean CAL quartiles

	Quartiles of Mean CAL				
	Q1 (n=296)	**Q2 (n=304)**	**Q3 (n=302)**	**Q4 (n=304)**	***P* value**
Age at enrollment					
<70 years	200 (67.57)	211 (69.41)	207 (68.54)	184 (60.53)	0.0806
≥70 years	96 (32.43)	93 (30.59)	95 (31.46)	120 (39.47)	
Total family income					
<$50,000	180 (60.81)	180 (59.21)	187 (61.92)	209 (68.75)	0.0762
≥$50,000	116 (39.19)	124 (40.79)	115 (38.08)	95 (31.25)	
Education					
High School	50 (17.24)	63 (20.93)	57 (19.32)	79 (26.42)	0.0807
College	126 (43.45)	135 (44.85)	136 (46.10)	133 (44.48)	
Post-College	114 (39.31)	103 (34.22)	102 (34.58)	87 (29.10)	
BMI					
Underweight/ Normal (< 24.9 kg/m^2)	124 (41.89)	147 (48.36)	140 (46.36)	130 (42.76)	0.2129
Overweight (25.0- 29.9 kg/m^2)	104 (35.14)	97 (31.91)	111 (36.75)	99 (32.57)	
Obese (≥30.0 kg/m^2)	68 (22.97)	60 (19.74)	51 (16.89)	75 (24.67)	
Cigarette smoking					
Never	178 (60.14)	182 (59.87)	139 (46.03)	136 (44.74)	<.0001
Former	110 (37.16)	118 (38.82)	155 (51.32)	148 (48.68)	
Current	8 (2.70)	4 (1.32)	8 (2.65)	20 (6.58)	
Birth control usage	104 (35.14)	126 (41.45)	115 (38.08)	111 (36.51)	0.4173
Cardiac therapeutic usage	10 (3.38)	5 (1.65)	5 (1.67)	9 (2.97)	0.3870

Table 4.5 Population continuous characteristics for WHI OsteoPerio ancillary study participants by Mean CAL quartiles

	Quartiles of Mean CAL				
	Q1 (n=296)	**Q2 (n=304)**	**Q3 (n=302)**	**Q4 (n=304)**	***P* value**
Age at enrollment, mean±SD, y	66.20±6.83	65.91±7.03	66.06±7.10	67.72±6.75	0.0040
Physical activity, mean±SD, MET hours per week	16.08±17.01	13.61±12.46	15.33±14.68	12.83±12.56	0.0214
Diastolic BP, mean±SD, mmHg	70.35±9.12	71.16±8.68	70.74±8.73	70.87±9.30	0.7444
Caffeine intake, mean±SD, dietary caffeine mg	156.02±111.26	158.46±119.46	183.29±139.53	173.25±135.47	0.0314
Alcohol intake, mean±SD, servings per week	2.02±3.55	2.81±4.56	3.01±5.43	2.89±5.14	0.0542

Table 4.6 Population microbial community characteristics for WHI OsteoPerio ancillary study participants by Mean CAL quartiles

	Quartiles of Mean CAL				
	Q1 (n=296)	**Q2 (n=304)**	**Q3 (n=302)**	**Q4 (n=304)**	***P* value**
Bacterial sp., No. (%)					
S. sanguis	165 (55.74)	173 (56.91)	193 (63.91)	182 (59.87)	0.1758
P. intermedia	126 (42.57)	111 (36.51)	132 (43.71)	146 (48.03)	0.0383
T. forsythensis	81 (27.36)	98 (32.24)	127 (42.05)	147 (48.36)	<.0001
Capnocytophaga sp.	108 (36.49)	97 (31.91)	127 (42.05)	118 (38.82)	0.0695
E. saburreum	81 (27.36)	82 (26.97)	112 (37.09)	121 (39.80)	0.0005
C. rectus	41 (13.85)	42 (13.82)	53 (17.55)	66 (21.71)	0.0272
P. gingivalis	32 (10.81)	33 (10.86)	43 (14.24)	67 (22.04)	0.0001
F. nucleatum	35 (11.82)	39 (12.83)	43 (14.24)	51 (16.78)	0.3234

Table 4.7 Population categorical characteristics for WHI OsteoPerio ancillary study participants by severity of Group 1 bacterial infection

	Group 1 Bacterial Infection			
	None/mild (n=322)	**Moderate (n=688)**	**Severe (n=196)**	***P* value**
Age at enrollment				
<70 years	575 (67.65)	181 (65.11)	46 (58.97)	0.2558
≥70 years	275 (32.35)	97 (34.89)	32 (41.03)	
Total family income				
<$50,000	519 (61.06)	184 (66.19)	53 (67.95)	0.1880
≥$50,000	331 (38.94)	94 (33.81)	25 (32.05)	
Education				
High School	172 (20.57)	57 (20.88)	20 (26.32)	0.1991
College	363 (43.42)	136 (49.82)	31 (40.79)	
Post-College	301 (36.00)	80 (29.30)	25 (32.89)	
BMI				
Underweight/ Normal (< 24.9 kg/m^2)	406 (47.76)	110 (39.57)	25 (32.05)	0.0148
Overweight (25.0- 29.9 kg/m^2)	273 (32.12)	102 (36.69)	36 (46.15)	
Obese (≥30.0 kg/m^2)	171 (20.12)	66 (23.74)	17 (21.79)	
Cigarette smoking				
Never	471 (55.41)	124 (44.60)	40 (51.28)	0.0365
Former	353 (41.53)	142 (51.08)	36 (46.15)	
Current	26 (3.06)	12 (4.32)	2 (2.56)	
Birth control usage	331 (38.94)	102 (36.69)	23 (29.49)	0.2336
Cardiac therapeutic usage	17 (2.00)	9 (3.26)	3 (3.85)	0.3458
Mean CAL				
Q1	231 (27.18)	54 (19.42)	11 (14.10)	<.0001
Q2	234 (27.53)	56 (20.14)	14 (17.95)	
Q3	201 (23.65)	82 (29.50)	19 (24.36)	
Q4	184 (21.65)	86 (30.94)	34 (43.59)	

Table 4.8 Population continuous characteristics for WHI OsteoPerio ancillary study participants by severity of Group 1 bacterial infection

	Group 1 Bacterial Infection			
	None/mild (n=322)	**Moderate (n=688)**	**Severe (n=196)**	***P* value**
Age at enrollment, mean±SD, y	66.26±7.04	66.47±6.81	68.85±6.17	0.069
Physical activity, mean±SD, MET hours per week	14.74±14.36	14.08±14.28	12.46±13.83	0.3701
Diastolic BP, mean±SD, mmHg	70.76±9.02	70.79±8.81	71.04±8.85	0.9668
Caffeine intake, mean±SD, dietary caffeine mg	162.97±122.44	172.86±129.40	202.32±163.58	0.0265
Alcohol intake, mean±SD, servings per week	2.59±4.51	2.96±5.52	2.78±4.19	0.5246

Table 4.9 Population categorical characteristics for WHI OsteoPerio ancillary study participants by severity of Group 2 bacterial infection

	Group 2 Bacteria			
	0 (n=610)	**1 (n=346)**	**2 (n=250)**	***P* value**
Age at enrollment				
<70 years	411 (67.38)	221 (63.87)	170 (68.00)	0.4641
≥70 years	199 (32.62)	125 (36.13)	80 (32.00)	
Total family income				
<$50,000	383 (62.79)	213 (61.56)	160 (64.00)	0.8292
≥$50,000	227 (37.21)	133 (38.44)	90 (36.00)	
Education				
High School	129 (21.68)	75 (21.87)	45 (18.22)	0.4114
College	264 (44.37)	160 (46.65)	106 (42.91)	
Post-College	202 (33.95)	108 (31.49)	96 (38.87)	
BMI				
Underweight/ Normal (< 24.9 kg/m^2)	295 (48.36)	148 (42.77)	98 (39.20)	0.0185
Overweight (25.0- 29.9 kg/m^2)	185 (30.33)	121 (34.97)	105 (42.00)	
Obese (≥30.0 kg/m^2)	130 (21.31)	77 (22.25)	47 (18.80)	
Cigarette smoking				
Never	319 (52.30)	180 (52.02)	136 (54.40)	0.6121
Former	266 (43.61)	157 (45.38)	108 (43.20)	
Current	25 (4.10)	9 (2.60)	6 (2.40)	
Birth control usage	237 (38.85)	125 (36.13)	94 (37.60)	0.7035
Cardiac therapeutic usage	12 (1.98)	10 (2.90)	7 (2.80)	0.6081
Mean CAL				
Q1	160 (26.23)	83 (23.99)	53 (21.20)	0.0047
Q2	171 (28.03)	87 (25.14)	46 (18.40)	
Q3	143 (23.44)	79 (22.83)	80 (32.00)	
Q4	136 (22.30)	97 (28.03)	71 (28.40)	

Table 4.10 Population continuous characteristics for WHI OsteoPerio ancillary study participants by severity of Group 2 bacterial infection

	Group 2 Bacteria			
	0 (n=610)	**1 (n=346)**	**2 (n=250)**	***P* value**
Age at enrollment, mean±SD, y	66.39±6.94	66.60±7.09	66.50±6.84	0.8975
Physical activity, mean±SD, MET hours per week	14.73±14.59	13.97±13.83	14.40±14.32	0.7372
Diastolic BP, mean±SD, mmHg	70.79±8.71	70.92±9.06	70.57±9.42	0.8922
Caffeine intake, mean±SD, dietary caffeine mg	167.33±125.68	160.68±118.84	179.12±142.10	0.2255
Alcohol intake, mean±SD, servings per week	2.74±5.10	2.33±3.87	3.07±4.95	0.1674

Table 4.11 Unadjusted and adjusted odds of suffering from migraines by exposure status for WHI OsteoPerio ancillary study participants

	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)[a]	*P* value
CDC/AAP Definition of Periodontal Disease[b]				
Severe/Moderate Periodontal Disease	0.812 (0.544, 1.213)	0.3097	0.811 (0.530, 1.241)	0.3343
Mean CAL[c]				
Mean CAL Q2	1.109 (0.673, 1.827)	0.6850	1.071 (0.628, 1.828)	0.8012
Mean CAL Q3	1.746 (1.000, 3.048)	0.0500	1.638 (0.904, 2.966)	0.1038
Mean CAL Q4	1.065 (0.648, 1.749)	0.8035	0.930 (0.546, 1.584)	0.7900
Infection with Group 1 Bacteria[d]				
Moderate Infection	1.622 (0.992, 2.652)	0.0540	1.565 (0.922, 2.658)	0.0973
Severe Infection	1.589 (0.673, 3.754)	0.2907	1.385 (0.547, 3.505)	0.4922
S. sanguis	0.904 (0.620, 1.320)	0.6025	0.927 (0.623, 1.379)	0.7073
P. intermedia	0.960 (0.662, 1.392)	0.8304	0.972 (0.656, 1.439)	0.8856
T. forsythensis	1.414 (0.950, 2.102)	0.0874	1.337 (0.720, 2.481)	0.3579
C. rectus	0.963 (0.592, 1.567)	0.8791	0.968 (0.583, 1.605)	0.8982
P. gingivalis	2.109 (1.082, 4.110)	0.0283	2.252 (1.121, 4.526)	0.0226
F. nucleatum	1.466 (0.806, 2.669)	0.2104	1.337 (0.720, 2.481)	0.3579
Infection with Group 2 Bacteria[e]				
Infection with 1 Group 2 Bacteria	0.821 (0.545, 1.236)	0.3447	0.776 (0.498, 1.207)	0.2599
Infection with 2 Group 2 Bacteria	1.455 (0.852, 2.484)	0.1699	1.363 (0.756, 2.458)	0.3036
Capnocytophaga sp.	1.115 (0.759, 1.637)	0.5803	1.159 (0.773, 1.738)	0.4758
E. saburreum	1.230 (0.822, 1.842)	0.3145	1.303 (0.853, 1.989)	0.2205

[a] **Adjusting for** age, education, income, BMI, smoking status, physical activity, history of birth control or cardiac therapeutic usage, blood pressure, and alcohol and caffeine consumption; [b] Reference group: No or mild periodontal disease; [c] Reference group: Mean CAL Q1; [d] Reference group: No or mild Group 1 bacterial infection; [e] Reference group: 0 Group 2 bacteria present

Table 4.12 Unadjusted and adjusted odds of suffering from mild headache symptoms by exposure status for WHI OsteoPerio ancillary study participants

	Crude OR (95% CI)	*P* value	Adjusted OR* (95% CI)	*P* value
CDC/AAP Definition of Periodontal Disease[b]				
Severe/Moderate Periodontal Disease	0.865 (0.699, 1.119)	0.2698	0.758 (0.336, 1.710)	0.5039
Mean CAL[c]				
Mean CAL Q2	1.015 (0.735, 1.402)	0.9288	0.986 (0.701, 1.387	0.9349
Mean CAL Q3	0.980 (0.708, 1.356)	0.9036	1.040 (0.737, 1.467)	0.8248
Mean CAL Q4	0.818 (0.591, 1.131)	0.2239	0.838 (0.593, 1.185)	0.3186
Infection with Group 1 Bacteria[d]				
Moderate Infection	0.855 (0.649,1.125)	0.2642	0.844 (0.623,1.144)	0.2751
Severe Infection	0.801 (0.499,1.287)	0.3591	0.855 (0.527,1.488)	0.6448
S. sanguis	1.124 (0.891,1.418)	0.3251	1.104 (0.864,1.410)	0.4282
P. intermedia	0.848 (0.673,1.069)	0.1631	0.831 (0.650,1.061)	0.1380
T. forsythensis	0.915 (0.723,1.158)	0.4606	0.993 (0.772,1.277)	0.9566
C. rectus	0.867 (0.638,1.178)	0.3614	0.814 (0.590,1.122)	0.7975
P. gingivalis	0.791 (0.570,1.096)	0.1591	0.850 (0.602,1.198)	0.3527
F. nucleatum	0.913 (0.656,1.272)	0.5921	1.028 (0.723,1.463)	0.8758
Infection with Group 2 Bacteria[e]				
Infection with 1 Group 2 Bacteria	0.986 (0.755,1.287)	0.9161	1.016 (0.764,1.352)	0.9109
Infection with 2 Group 2 Bacteria	0.902 (0.670,1.215)	0.4983	0.973 (0.695,1.363)	0.8752
Capnocytophaga sp.	0.902 (0.712,1.142)	0.3908	0.907 (0.708,1.163)	0.4426
E. saburreum	0.979 (0.768,1.248)	0.8628	0.977 (0.756,1.261)	0.8569

[a] **Adjusting for** age, education, income, BMI, smoking status, physical activity, history of birth control or cardiac therapeutic usage, blood pressure, and alcohol and caffeine consumption; [b] Reference group: No or mild periodontal disease; [c] Reference group: Mean CAL Q1; [d] Reference group: No or mild Group 1 bacterial infection; [e] Reference group: 0 Group 2 bacteria present

Table 4.13 Unadjusted and adjusted odds of suffering from moderate headache symptoms by exposure status for WHI OsteoPerio ancillary study participants

	Crude OR (95% CI)	*P* value	Adjusted OR* (95% CI)	*P* value
CDC/AAP Definition of Periodontal Disease[b]				
Severe/Moderate Periodontal Disease	0.737 (0.497,1 .091)	0.1276	0.728 (0.483, 1.098)	0.1299
Mean CAL[c]				
Mean CAL Q2	0.699 (0.402, 1.112)	0.1207	0.614 (0.356, 1.059)	0.0797
Mean CAL Q3	0.687 (0.413, 1.141)	0.1471	0.728 (0.427, 1.239)	0.2417
Mean CAL Q4	0.726 (0.440, 1.196)	0.2085	0.806 (0.479, 1.355)	0.4148
Infection with Group 1 Bacteria[d]				
Moderate Infection	0.875 (0.561, 1.366)	0.5579	0.819 (0.507, 1.322)	0.4138
Severe Infection	0.422 (0.151, 1.181)	0.1004	0.458 (0.161, 1.306)	0.1442
S. sanguis	1.138 (0.781, 1.657)	0.5018	1.092 (0.739, 1.615)	0.6589
P. intermedia	0.907 (0.625, 1.316)	0.6072	0.845 (0.571, 1.251)	0.4008
T. forsythensis	0.747 (0.506, 1.105)	0.1441	0.817 (0.543, 1.231)	0.3344
C. rectus	0.837 (0.501, 1.397)	0.4957	0.751 (0.436, 1.293)	0.3019
P. gingivalis	0.694 (0.389, 1.240)	0.2171	0.764 (0.424, 1.380)	0.3725
F. nucleatum	1.006 (0.594, 1.706)	0.9814	1.114 (0.643, 1.928)	0.7007
Infection with Group 2 Bacteria[e]				
Infection with 1 Group 2 Bacteria	1.672 (1.111, 2.516)	0.0137	1.753 (1.139, 2.697)	0.0108
Infection with 2 Group 2 Bacteria	1.051 (0.635, 1.740)	0.8473	1.168 (0.683, 1.998)	0.5697
Capnocytophaga sp.	1.217 (0.840, 1.765)	0.2997	1.229 (0.833, 3.409)	0.2986
E. saburreum	1.052 (0.715, 1.547)	0.7980	1.090 (0.730, 1.628)	0.6732

[a] **Adjusting for** age, education, income, BMI, smoking status, physical activity, history of birth control or cardiac therapeutic usage, blood pressure, and alcohol and caffeine consumption; [b] Reference group: No or mild periodontal disease; [c] Reference group: Mean CAL Q1; [d] Reference group: No or mild Group 1 bacterial infection; [e] Reference group: 0 Group 2 bacteria present

Table 4.14 Unadjusted and adjusted odds of suffering from severe headache symptoms by exposure status for WHI OsteoPerio ancillary study participants

	Crude OR (95% CI)	*P* value	Adjusted OR* (95% CI)	*P* value
CDC/AAP Definition of Periodontal Disease[b]				
Severe/Moderate Periodontal Disease	0.768 (0.358,1.650)	0.4988	0.758 (0.336,1.710)	0.5039
Mean CAL[c]				
Mean CAL Q2	0.518 (0.189,1.419)	0.2005	0.510 (0.168,1.544)	0.2334
Mean CAL Q3	0.687 (0.413,1.141)	0.1471	0.535 (0.176,1.622)	0.2688
Mean CAL Q4	0.700 (0.277,1.765)	0.4494	0.858 (0.325,2.265)	0.3186
Infection with Group 1 Bacteria[d]				
Moderate Infection	0.608 (0.231,1.605)	0.3152	0.726 (0.269,1.956)	0.5265
Severe Infection	0.431 (0.058,3.229)	0.4131	0.530 (0.070,4.024)	0.5389
S. sanguis	0.727 (0.356,1.484)	0.3810	0.641 (0.298,1.380)	0.2559
P. intermedia	0.741 (0.352,1.560)	0.4299	0.783 (0.354,1.730)	0.5451
T. forsythensis	0.670 (0.306,1.468)	0.3170	0.871 (0.387,1.964)	0.7399
C. rectus	1.190 (0.482,2.940)	0.7061	1.361 (0.540,3.427)	0.5135
P. gingivalis	0.626 (0.188,2.083)	0.4454	0.745 (0.221,2.512)	0.6353
F. nucleatum	0.917 (0.317,2.654)	0.8726	1.152 (0.392,3.385)	0.7975
Infection with Group 2 Bacteria[e]				
Infection with 1 Group 2 Bacteria	2.856 (1.281,6.368)	0.0103	3.169 (1.310,7.663)	0.0105
Infection with 2 Group 2 Bacteria	1.202 (0.407,3.555)	0.7388	1.578 (0.507,4.910)	0.4312
Capnocytophaga sp.	1.570 (0.768,3.207)	0.2161	1.583 (0.735,3.409)	0.2403
E. saburreum	1.114 (0.529,2.350)	0.7758	1.337 (0.613,2.918)	0.4659

[a] **Adjusting for** age, education, income, BMI, smoking status, physical activity, history of birth control or cardiac therapeutic usage, blood pressure, and alcohol and caffeine consumption; [b] Reference group: No or mild periodontal disease; [c] Reference group: Mean CAL Q1; [d] Reference group: No or mild Group 1 bacterial infection; [e] Reference group: 0 Group 2 bacteria present

CHAPTER 5

CONCLUSIONS

5.1 Discussion:

5.1.1 Migraines

Periodontal disease, as defined by the CDC/AAP and by quartiles of mean CAL, was not significantly associated with migraines among older, predominantly white women in the US. The Fifth European Workshop on Periodontology, hosted in 2005, used CAL as the sole marker of periodontitis in epidemiologic studies of risk factors. In 2007, the CDC/AAP proposed the first draft of the rankings used to address the first hypothesis of Aim 1. The current rankings included cases definitions for three instead of two rankings. Since then, the rankings set by the CDC/AAP have been used in numerous epidemiological studies (Page & Eke, 2007; Penoni, Torres, Farias, & Fernandes, 2016) and it has even been suggested that these rankings could be standardized for all future population-based studies of periodontal disease (Page & Eke, 2007).

However, these definitions are based on measurements of periodontal destruction (PD and CAL), which capture the damage caused by inflammation and are not indicative of a current inflammatory response. Furthermore, both PD and CAL may lead to misclassification as using PD might result in pseudo-pocket

to be considered true and using CAL may similarly cause sites of gingival recession by mechanical forces to be considered true symptoms of periodontal disease (Pei, He, & Ouyang, 2012). Because we are suggesting that the inflammatory responses related to a current periodontal infection are related to migraines, the association would likely be underestimated by these exposure measures as they can lead to an overestimation of periodontal disease presence. Conversely, using PD and CAL as measures of periodontal disease can also lead to an underestimation of periodontal disease prevalence, as both measures only consider the interproximal sites of teeth and periodontal disease can also occur at buccal and lingual sites. To combat these issues, Pei and colleagues proposed a three-level definition of periodontal disease that uses PD, CAL, and additionally measure, bleeding on probing (BOP), to define the severity of disease where all six sites per tooth were considered (Pei et al., 2012). A positive BOP would be indicative of a current infection and its subsequent inflammatory responses. Future investigations into the relationship between periodontal disease and migraines would benefit from using this definition of periodontal disease. Because of this potential misclassification bias, our study may have failed to detect meaningful differences between those with and without periodontal disease. Future investigations into the relationship between periodontal disease and migraines should consider a more inclusive definition that accounts for inflammation.

Peskersoy and colleagues reported findings from their investigation into the relationship between migraines and oral comorbidities. They found that

participants with migraines reported more frequently tooth wear and abrasion. Furthermore, they found that two additional measures of oral health, DMFT and plaque index scores were significantly associated with migraines. While this is evidence of a relationship between oral health and migraines, this study did not use any of the measures or definitions of periodontal disease used in this study, making comparisons between their study and ours impossible.

Participants with Group 1 bacteria present reported migraine more frequently (Moderate infection adjusted OR = 1.565, 95% CI: 0.922, 2.658; severe infection adjusted OR = 1.385, 95% CI: 0.547, 3.505), even though this did not reach statistical significance. However, the presence of *P. gingivalis* was positively associated with migraines (adjusted OR = 2.252, 95% CI: 1.121, 4.526). As a prominent member of the oral microbiome and the demonstrated ability to colonize the oral epithelial, *P. gingivalis* is considered a keystone species in periodontal disease as a member of the “red complex” identified by Haffajee and colleagues, which has been strongly implicated in periodontal disease (Haffajee, Socransky, Patel, & Song, 2008). As part of the immune response to periodontal disease, the immune system produces the antimicrobial agent, NO. The lipid A-associated proteins of the *P. gingivalis* LPS (PgLPS) has been shown to induce nitric oxide production in clinical studies (E. Y. Choi et al., 2007; Herath et al., 2016) and is considered a crucial virulence factor in periodontal pathogenesis. Our study provides evidence of a significant, positive association between a bacterial species, *P. gingivalis*, and migraines.

Gonzalez and colleagues, in a collaboration with Knight labs, were the first to suggest a relationship between oral bacteria and migraines (Gonzalez et al., 2016). They reported significant increases in nitrate, nitrite, and nitric oxide reductase genes in oral samples collected from those who suffer from migraines. After claiming they had evidence of a potential link between oral microbes and migraines, the results of this study were sensationalized by numerous national news sources. A criticism of this study was that the authors implied that they directly measured genes of interests related to reducing nitrates and nitrites. However, their predictions were based on prior projects, and did not directly demonstrate link between periodontal bacteria, nitric oxide production, and migraines. Additionally, none of their methods or results suggested any consideration for important confounders. Infection with Group 2 bacteria was not associated with migraines.

5.1.2 Headache Disorders

Periodontal disease, defined two ways, was not associated with headache disorders. Furthermore, headaches were not related to the presence of Group 1 bacteria. We hypothesized that the presence of NO producing oral bacteria (Group 1) would be positively associated with migraines. As headaches have many different causes, the association of Group 1 bacteria and headaches would not have expected to be strong. Without differentiating the type of headache disorder, it is impossible to limit our exposure population to only those headache disorders linking to NO.

Contradictory to our second hypothesis of Aim 2, Group 2 bacteria were positively associated with headache disorders. Infection with a Group 2 bacterium was significantly associated with moderate and severe headache symptoms (Moderate symptom adjusted OR = 1.753; 95% CI: 1.139,2.697; Severe symptom adjusted OR = 3.169, 95% CI: 1.310,7.663); however, no individual species of Group 2 bacteria was associated with migraines.

As periodontal disease and its associated bacterial pathogens have been shown to induce NO production by the immune system (Eun Young Choi et al., 2011; Kim, Ha, Choi, Choi, & Choi, 2004; Pelt, Zimmermann, Ulbrich, & Bernimoulin, 2002; Skaleric, Gaspirc, McCartney-Francis, Masera, & Wahl, 2006; Hussain, Mckay, Gonzales-Marin, & Allaker, 2015; E. Y. Choi et al., 2007; Herath et al., 2016; Kato, Mikami, & Saito, 2001; Velsko et al., 2015; Chukkapalli et al., 2015; English, Patrick, Orlicek, McCordic, & Shenep, 1996; Jian Jun Gao, Xue, Zuvanich, Haghi, & Morrison, 2001; Kendall, Haase, Li, Xiao, & Bartold, 2000; Hirose et al. 2001), and NO has been implicated in the pathophysiology of headache disorders (D'andrea et al., 1994; Gruber et al., 2010; Olesen, 2008; Sarchielli et al., 1996; Shimomura et al., 1999), it is biologically feasible that periodontal disease and its associated bacterial pathogens to be positively associated with headache disorders.

Additionally, it is important to note, that estrogen has been shown to be protective against periodontal disease (Brasil et al., 2017; Nebel, 2012), but is associated with headache disorders, and migraines in particular (Chai, Peterlin, & Calhoun, 2014). Estrogen plays an important role in regulating inflammatory

responses to infection, specifically the production of NO (Brasil et al., 2017; Nevzati et al., 2015; Townsend, Meuchel, Thompson, Pabelick, & Prakash, 2011). Estrogen receptors (ER), ERα and ERβ are expressed in smooth muscle cells, macrophages, and endothelial cells, and activation of ERα in endothelial cells has shown to increase the expression of NO synthase (eNOS) in the presence of estrogen (17β-estradiol) (Furchgott, 1999; Mendelsohn, 2000; Chen et al., 1999). Furthermore, mutations in the ERα gene have been associated with increased risk of periodontal disease (Weng et al., 2015). The ability of ERs to increase NO production in the presence of estrogen could explain the increased prevalence of headache disorders in women (Chai et al., 2014)

Findings from our investigation into the relationship between periodontal disease and the subsequent production of NO due to its related pathogenic bacteria and headache disorders provides some evidence of the association between our exposures of interest and migraines but not between our general headache disorder variable. Further investigations into the periodontal disease and headache disorders is warranted.

5.2 Strengths:

This study has several strengths. First, the WHI ancillary OsteoPerio study had information on all important, potential confounders in the relationship between periodontal disease and its ensuing inflammatory responses and headache disorders. This allowed for statistical control of potential confounders. Second, the variables from the OsteoPerio study were carefully chosen, collected, and measured. Third, a biologically plausible hypothesis linking NO

producing oral bacteria and migraines was tested. Lastly, my study these findings contribute to an understudied area on headache etiology.

5.3 Limitations:

First, self-reported headache is vulnerable to misclassification bias. A recent validation study found high concordance between self-reported questionnaire data and medical records (κ=.61 for chronic conditions), suggesting our study would only minimally be influenced by misclassification bias (Van Gelder et al., 2015). Second, the bacterial species were classified as present or absent with no quantification limiting the statistical analyses at our disposal. Third, the results of our study are not generalizable to the entire United States population, but to older women aged 53 to 83. These results need to be replicated in other populations.

Last, this study used a cross-sectional design and therefore temporality cannot be assessed. This is particularly problematic since headache disorders are more prevalent amongst younger individuals (Younger, 2016), and conversely periodontal disease, as defined by periodontal destruction, is typically more prevalent in older populations and includes past and current infection (Leite, Peres, Do, Demarco, & Peres, 2017; Page & Eke, 2007). However, the proposed biological plausibility explains this perceived paradox. NO produced by the immune system in response periodontal disease causes the vasodilation responsible for the pain suffered from headache disorders. It is well established that the immune system declines with age, including the production of NO (Kissin, Tomasi, McCartney-Francis, Gibbs, & Smith, 1997; Liang, Domon,

Hosur, Wang, & Hajishengallis, 2009). If our hypotheses are correct, the aging body's declining ability to illicit an immune response, specifically the production of NO, not only explains the body's decreased ability to fight the infections associated with periodontal disease, but also explains the decrease headache disorder prevalence in older populations. Furthermore, results from the National Health and Nutrition Examination Survey (NHANES) 2009-2012 suggests that almost half of adults age 30 years and older have periodontal disease with severe periodontal disease affecting 8.9% of the population (Eke et al., 2015). Moreover, NHANES III data suggest 90% of persons aged 20-79 needed periodontal treatment with increasing complexity of periodontal treatments with age (Dye & Vargas, 2002). While periodontal disease is associated with older age, children and adolescence also suffer (Merchant et al., 2015).

5.4 Importance:

Headaches are the 6th leading cause of disability globally. Approximately 46% of the adult population suffers from headaches, 42% suffer from tension-type headaches, 11% suffer from migraines, and 3% suffer from chronic daily headaches (Stovner et al., 2007). Considering the disability induced by headache disorders, further investigation into their cause is warranted. The literature on the association between periodontal disease and headache disorders is limited, even though it is biologically plausible. Our study provides epidemiological data on the association between periodontal disease and headache disorders.

5.5 Conclusions:

P. gingivalis presence is positively associated with migraines among older white women after controlling for potential confounders. Further investigation into measures of oral health, oral microbes, and NO in relation to headache disorders is warranted.

REFERENCES:

Aamodt, A. H., Stovner, L. J., Midthjell, K., Hagen, K., & Zwart, J. A. (2007). Headache prevalence related to diabetes mellitus. The Head-HUNT Study. European Journal of Neurology, 14(7), 738–744. http://doi.org/10.1111/j.1468-1331.2007.01765.x

American Academy of Periodontology. (2015). American Academy of Periodontology Task Force Report on the Update to the 1999 Classification of Periodontal. Journal of Periodontology, 86(7), 835–838. http://doi.org/10.1902/jop.2015.157001

Bagdy, G., Riba, P., Kecskeméti, V., Chase, D., & Juhász, G. (2010). Headache-type adverse effects of NO donors: Vasodilation and beyond. British Journal of Pharmacology, 160(1), 20–35. http://doi.org/10.1111/j.1476-5381.2010.00643.x

Bond, D. S., Roth, J., Nash, J. M., & Wing, R. R. (2011). Migraine and obesity: Epidemiology, possible mechanisms and the potential role of weight loss treatment. Obesity Reviews, 12(501). http://doi.org/10.1111/j.1467-789X.2010.00791.x

Borkum, J. M. (2015). Migraine Triggers and Oxidative Stress: A Narrative Review and Synthesis. Headache, 56(1), 12–35. http://doi.org/10.1111/head.12725

Brasil, S. C., Santos, R. M. M., Fernandes, A., Alves, F. R. F., Pires, F. R., Siqueira, J. F. J., & Armada, L. (2017). Influence of estrogen deficiency on the development of apical periodontitis. International Endodontic Journal, 50(2), 161–166. http://doi.org/10.1111/iej.12612

Brennan, R. M., Genco, R. J., Wilding, G. E., Hovey, K. M., Trevisan, M., & Wactawski-Wende, J. (2007). Bacterial Species in Subgingival Plaque and Oral Bone Loss in Postmenopausal Women. Journal of Periodontology, 78(6), 1051–1061. http://doi.org/10.1902/jop.2007.060436

Buse, D. C., Silberstein, S. D., Manack, A. N., Papapetropoulos, S., & Lipton, R. B. (2013). Psychiatric comorbidities of episodic and chronic migraine. Journal of Neurology, 260(8), 1960–1969. http://doi.org/10.1007/s00415-012-6725-x

Cappy, H., Lucas, C., Catteau-Jonard, S., & Robin, G. (2015). Migraine et contraception. Gynecologie Obstetrique Fertilite, 43(3), 234–241. http://doi.org/10.1016/j.gyobfe.2015.01.006

Chai, N. C., Peterlin, B. L., & Calhoun, A. H. (2014). Migraine and estrogen. Current Opinion in Neurology, 27(3), 315–24. http://doi.org/10.1097/WCO.0000000000000091

Cho, I., & Blaser, M. J. (2012). The human microbiome: at the interface of health and disease. Nat Rev Genet, 13(4), 260–270. Retrieved from http://dx.doi.org/10.1038/nrg3182

Choi, E. Y., Hwang, Y. M., Lee, J. Y., Choi, J. I., Choi, I. S., Jin, J. Y., … Kim, S. J. (2007). Lipid A-associated proteins from Porphyromonas gingivalis stimulate release of nitric oxide by inducing expression of inducible nitric oxide synthase. Journal of Periodontal Research, 42(4), 350–360. http://doi.org/10.1111/j.1600-0765.2006.00956.x

Choi, E. Y., Jin, J. Y., Lee, J. Y., Choi, J. Il, Choi, I. S., & Kim, S. J. (2011). Melatonin inhibits Prevotella intermedia lipopolysaccharide-induced production of nitric oxide and interleukin-6 in murine macrophages by suppressing NF-kB and STAT1 activity. Journal of Pineal Research, 50(2), 197–206. http://doi.org/10.1111/j.1600-079X.2010.00829.x

Chukkapalli, S. S., Rivera-Kweh, M. F., Velsko, I. M., Chen, H., Zheng, D., Bhattacharyya, I., … Kesavalu, L. (2015). Chronic oral infection with major periodontal bacteria Tannerella forsythia modulates systemic atherosclerosis risk factors and inflammatory markers. Pathogens and Disease, 73(3), 1–12. http://doi.org/10.1093/femspd/ftv009

D'andrea, G., Welch, K., Perini, F., Alecci, M., Zamberlan, F., & Hasselmark, L. (1994). Decreased collagen-induced platelet aggregation and increased platelet arginine levels in migraine: A possible link with the NO pathway. Cephalalgia, 14(5), 352–356. http://doi.org/10.1046/j.1468-2982.1994.1405352.x

Dye, B. A., & Vargas, C. M. (2002). The use of a modified CPITN approach to estimate periodontal treatment needs among adults aged 20-79 years by socio-demographic characteristics in the United States, 1988-94. Community Dental Health, 19(4), 215–223.

Eke, P. I., Dye, B. A., Wei, L., Slade, G. D., Thornton-Evans, G. O., Borgnakke, W. S., … Genco, R. J. (2015). Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. Journal of Periodontology, 86(5), 611–622. http://doi.org/10.1902/jop.2015.140520

English, B. K., Patrick, C. C., Orlicek, S. L., McCordic, R., & Shenep, J. L. (1996). Lipoteichoic acid from viridans streptococci induces the production of tumor necrosis factor and nitric oxide by murine macrophages. Journal of Infectious Diseases, 174(6), 1348–1351.

Fagernæs, C. F., Heuch, I., Zwart, J., Winsvold, B. S., Linde, M., & Hagen, K. (2015). Blood pressure as a risk factor for headache and migraine: A prospective population-based study. European Journal of Neurology, 22(1), 156–162. http://doi.org/10.1111/ene.12547

Gonzalez, A., Hyde, E., Sangwan, N., Gilbert, J. A., Viirre, E., & Knight, R. (2016). Migraines Are Correlated with Higher Levels of Nitrate-, Nitrite-, and Nitric Oxide-Reducing Oral Microbes in the American Gut Project Cohort. mSystems, 1(5), 3–6. http://doi.org/10.1128/mSystems.00105-16.Editor

Gruber, H. J., Bernecker, C., Lechner, A., Weiss, S., Wallner-Blazek, M., Meinitzer, A., … Truschnig-Wilders, M. (2010). Increased nitric oxide stress is associated with migraine. Cephalalgia, 30(4), 486–492. http://doi.org/10.1111/j.1468-2982.2009.01964.x

Haffajee, A. D., Japlit, M., Bogren, A., Kent, R. L., Goodson, J. M., & Socransky, S. S. (2005). Differences in the subgingival microbiota of Swedish and USA subjects who were periodontally healthy or exhibited minimal periodontal disease. Journal of Clinical Periodontology, 32(1), 33–39. http://doi.org/10.1111/j.1600-051X.2004.00624.x

Hagen, K., Vatten, L., Stovner, L. J., Zwart, J. A., Krokstad, S., & Bovim, G. (2002). Low socio-economic status is associated with increased risk of frequent headache: A prospective study of 22 718 adults in Norway. Cephalalgia, 22(8), 672–679. http://doi.org/10.1046/j.1468-2982.2002.00413.x

Heckman, B. D., & Britton, A. J. (2015). Headache in African Americans: An Overlooked Disparity. Journal of the National Medical Association, 107(2), 39–45. http://doi.org/10.1016/S0027-9684(15)30023-7

Herath, T. D. K., Darveau, R. P., Seneviratne, C. J., Wang, C.-Y., Wang, Y., & Jin, L. (2016). Heterogeneous Porphyromonas gingivalis LPS modulates immuno-inflammatory response, antioxidant defense and cytoskeletal dynamics in human gingival fibroblasts. Scientific Reports, 6(August), 29829. http://doi.org/10.1038/srep29829

Hoffman, M., & McCulloch, T. (2013). Relief from cluster headaches following extraction of an ipsilateral infected tooth. ENT Journal, 92(6), 264–268.

Hussain, Q. A., Mckay, I. J., Gonzales-Marin, C., & Allaker, R. P. (2015).

Regulation of adrenomedullin and nitric oxide production by periodontal bacteria. Journal of Periodontal Research, 50(5), 650–657. http://doi.org/10.1111/jre.12246

Jian Jun Gao, Xue, Q., Zuvanich, E. G., Haghi, K. R., & Morrison, D. C. (2001). Commercial preparations of lipoteichoic acid contain endotoxin that contributes to activation of mouse macrophages in vitro. Infection and Immunity, 69(2), 751–757. http://doi.org/10.1128/IAI.69.2.751-757.2001

Kato, C., Mikami, M., & Saito, K. (2001). Nitric Oxide Production and iNOS mRNA Expression in Mice Induced by Repeated Stimulation with Live Fusobacterium nucleatum, 45(1), 69–78.

Kendall, H. K., Haase, H. R., Li, H., Xiao, Y., & Bartold, P. M. (2000). Nitric oxide synthase type-II is synthesized by human gingival tissue and cultured human gingival fibroblasts. Journal of Periodontal Research, 35(4), 194–200. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10983879

Kim, S. J., Ha, M. S., Choi, E. Y., Choi, J. Il, & Choi, I. S. (2004). Prevotella intermedia lipopolysaccharide stimulates release of nitric oxide by inducing expression of inducible nitric oxide synthase. Journal of Periodontal Research, 39(6), 424–431. http://doi.org/10.1111/j.1600-0765.2004.00757.x

Kissin, E., Tomasi, M., McCartney-Francis, N., Gibbs, C. L., & Smith, P. D. (1997). Age-related decline in murine macrophage production of nitric oxide. The Journal of Infectious Diseases, 175(4), 1004–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9086170

Leite, F. R. M., Peres, K. G., Do, L. G., Demarco, F. F., & Peres, M. A. (2017). Prediction of Periodontitis Occurrence: Influence of the Classification, Sociodemographic and General Health Information. Journal of Periodontology, 1–14. http://doi.org/10.1902/jop.2017.160607

Liang, S., Domon, H., Hosur, K. B., Wang, M., & Hajishengallis, G. (2009). Age-related alterations in innate immune receptor expression and ability of macrophages to respond to pathogen challenge in vitro. Mechanisms of Aging and Development, 130(8), 538–546. http://doi.org/10.1124/dmd.107.016501.CYP3A4-Mediated

Mancinelli, R. L., & McKay, C. P. (1983). Effects of nitric oxide and nitrogen dioxide on bacterial growth. Applied and Environmental Microbiology, 46(1), 198–202.

Martin, V. T., & Vij, B. (2016). Diet and Headache: Part 1. Headache Currents, 56(9), 1543–1552. http://doi.org/10.1111/head.12953

Merchant, A., Nahhas, G., Wadwa, R., Zhang, J., Tang, Y., Johnson, L., … Morrato, E. (2015). Periodontal Microorganisms and Cardiovascular Risk Markers in Youth With Type 1 Diabetes and No Diabetes. Journal of Periodontology, 87(4), 1–11. http://doi.org/10.1902/jop.2015.150531

Nebel, D. (2012). Functional importance of estrogen receptors in the periodontium. Swedish Dental Journal. Supplement, (221), 11–66.

Nevzati, E., Shafighi, M., Bakhtian, K. D., Treiber, H., Fandino, J., & Fathi, A. R. (2015). Estrogen induces nitric oxide production via nitric oxide synthase activation in endothelial cells. Acta Neurochirurgica. Supplement, 120, 141–145. http://doi.org/10.1007/978-3-319-04981-6_24

Olesen, J. (2008). The role of nitric oxide (NO) in migraine, tension-type headache and cluster headache. Pharmacology & Therapeutics, 120(2), 157–71. http://doi.org/10.1016/j.pharmthera.2008.08.003

Olesen, J. (2010). Nitric Oxide-Related Drug Targets in Headache. Neurotherapeutics, 7(2), 183–190. http://doi.org/10.1016/j.nurt.2010.03.006

Page, R. C., & Eke, P. I. (2007). Case Definitions for Use in Population-Based Surveillance of Periodontitis. The Journal of Periodontology, 78(7 Suppl), 1387–1399. http://doi.org/10.1902/jop.2007.060264

Pei, X. Y., He, L., & Ouyang, X. Y. (2012). using Probing Depth , Clinical Attachment Loss and Bleeding on Probing : Analysis of a Rural Chinese Population, 89–96. http://doi.org/10.3290/j.cjdr.a38273

Pelt, P., Zimmermann, B., Ulbrich, N., & Bernimoulin, J. P. (2002). Effects of lipopolysaccharide extracted from Prevotella intermedia on bone formation and on the release of osteolytic mediators by fetal mouse osteoblasts in vitro. Journal of Animal Science, 80(1), 859–866. http://doi.org/10.1016/S0003-9969(02)00150-4

Penoni, D. C., Torres, S. R., Farias, M. L. F., & Fernandes, T. M. (2016). Association of osteoporosis and bone medication with the periodontal condition in elderly women, 1887–1896. http://doi.org/10.1007/s00198-015-3437-y

Peskersoy, C., Peker, S., Kaya, A., Unalp, A., & Gokay, N. (2016). Evaluation of the relationship between migraine disorder and oral comorbidities: Multicenter randomized clinical trial. Turkish Journal of Medical Sciences, 46(3), 712–718. http://doi.org/10.3906/sag-1412-71

Sahli, M. W., Wactawski-Wende, J., Ram, P. K., LaMonte, M. J., Hovey, K. M., Genco, R. J., … Millen, A. E. (2015). Association of plasma 25-

hydroxyvitamin D concentrations and pathogenic oral bacteria in postmenopausal women. J Periodontol, 85(7), 944–955. http://doi.org/10.1902/jop.2013.130518

Sarchielli, P., Tognoloni, M., Russo, S., Vulcano, M., Feleppa, M., Mala, M., … Gallai, V. (1996). Variations in the platelet arginine/nitric oxide pathway during the ovarian cycle in females affected by menstrual migraine. Cephalalgia, 16, 168–475.

Scher, A. I., Terwindt, G. M., Picavet, H. S. J., Verschuren, W. M. M., Ferrari, M. D., & Launer, L. J. (2005). Cardiovascular risk factors and migraine: the GEM population-based study. Neurology, 64(4), 614–20. http://doi.org/10.1212/01.WNL.0000151857.43225.49

Shimomura, T., Murakami, F., Kotani, K., Ikawa, S., & Kono, S. (1999). Platelet nitric oxide metabolites in migraine. Cephalalgia, 19, 218–222.

Skaleric, U., Gaspirc, B., McCartney-Francis, N., Masera, A., & Wahl, S. M. (2006). Proinflammatory and antimicrobial nitric oxide in gingival fluid of diabetic patients with periodontal disease. Infection and Immunity, 74(12), 7010–7013. http://doi.org/10.1128/IAI.00071-06

Slade, G. D., Offenbacher, S., Beck, J. D., Heiss, G., & Pankow, J. S. (2000). Acute-phase Inflammatory Response to Periodontal Disease in the US population. Journal of Dental Research, 79(1), 49–57.

Socransky, S. S., Haffajee, a D., Cugini, M. a, Smith, C., & Kent, R. L. (1998). Microbial complexes in subgingival plaque. Journal of Clinical Periodontology, 25(2), 134–144. http://doi.org/10.1111/j.1600-051X.1998.tb02419.x

Steiner, T. J., Stovner, L. J., & Birbeck, G. L. (2013). Migraine: The seventh disabler. Headache, 14(1), 1–2. http://doi.org/10.1111/head.12034

Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Adair-Rohani, K.H., Amann, M., … Ezzati, M. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet, 380(9859), 2224–2260. http://doi.org/10.1016/S0140-6736(12)61766-8.A

Stovner, L. J., Hagen, K., Jensen, R., Katsarava, Z., Lipton, R. B., Scher, A. I., … Zwart, J. A. (2007). The global burden of headache: A documentation of headache prevalence and disability worldwide. Cephalalgia, 27(3), 193–210. http://doi.org/10.1111/j.1468-2982.2007.01288.x

Thadani, U., & Rodgers, T. (2006). Side effects of using nitrates to treat angina. Expert Opinion on Drug Safety, 5, 667–674. http://doi.org/10.1517/14740338.5.5.667

Townsend, E. A., Meuchel, L. W., Thompson, M. A., Pabelick, C. M., & Prakash, Y. S. (2011). Estrogen increases nitric-oxide production in human bronchial epithelium. The Journal of Pharmacology and Experimental Therapeutics, 339(3), 815–824. http://doi.org/10.1124/jpet.111.184416

Van Gelder, M. M. H. J., Schouten, N. P. E., Merkus, P. J. F. M., Verhaak, C. M., Roeleveld, N., & Roukema, J. (2015). Using web-based questionnaires and obstetric records to assess general health characteristics among pregnant women: A validation study. Journal of Medical Internet Research, 17(6), e149. http://doi.org/10.2196/jmir.3847

Varkey, E., Hagen, K., Zwart, J. A., & Linde, M. (2008). Physical activity and headache: Results from the Nord-Trondelag Health Study (HUNT). Cephalalgia, 28(12), 1292–1297. http://doi.org/10.1111/j.1468-2982.2008.01678.x

Velsko, I. M., Chukkapalli, S. S., Rivera-Kweh, M. F., Zheng, D., Aukhil, I., Lucas, A. R., … Kesavalu, L. (2015). Periodontal pathogens invade gingiva and aortic adventitia and elicit Iinflammasome activation in αvβ6 integrin-deficient mice. Infection and Immunity, 83(12), 4582–4593. http://doi.org/10.1128/IAI.01077-15

Weng, H., Zhang, C., Hu, Y. Y., Yuan, R. X., Zuo, H. X., Yan, J. Z., & Niu, Y. M. (2015). Association between Estrogen Receptor- α Gene XbaI and PvuII Polymorphisms and Periodontitis Susceptibility: A Meta-Analysis. Disease Markers, 2015. http://doi.org/10.1155/2015/741972

Younger, D. S. (2016). Epidemiology of Migraine. Neurologic Clinics, 34(4), 849–861. http://doi.org/10.1016/j.ncl.2016.06.011

World Headache Alliance (WHA). 2017. Retrieved from: http://www.w-h-a.